DESCRIPTION

MATERIALS AND METHODS FOR DETECTING, PREVENTING, AND TREATING RETROVIRAL INFECTION

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Cross-Reference to Related Application

This application claims the benefit of U.S. Provisional Application Serial No. 60/270,745, filed February 22, 2001.

Background of the Invention

Domestic cats are subject to infection by several retroviruses, including feline leukemia virus (FeLV), feline sarcoma virus (FeSV), endogenous type C oncoronavirus (RD-114), and feline syncytia-forming virus (FeSFV). Of these, FeLV is the most significant pathogen, causing diverse symptoms including lymphoreticular and myeloid neoplasms, anemias, immune-mediated disorders, and an immunodeficiency syndrome that is similar to human acquired immune deficiency syndrome (AIDS). Recently, a particular replication-defective FeLV mutant, designated FeLV-AIDS, has been more particularly associated with immunosuppressive properties.

The discovery of feline T-lymphotropic lentivirus (now designated as feline immunodeficiency virus, FIV) was first reported in Pedersen *et al.* (1987). Characteristics of FIV have been reported in Yamamoto *et al.* (1988a); Yamamoto *et al.* (1988b); and Ackley *et al.* (1990). Seroepidemiologic data have shown that infection by FIV is indigenous to domestic and wild felines throughout the world. A wide variety of symptoms are associated with infection by FIV, including abortion, alopecia, anemia, conjunctivitis, chronic

rhinitis, enteritis, gingivitis, hematochezia, neurologic abnormalities, periodontitis, and seborrheic dermatitis. The immunologic hallmark of domestic cats infected with FIV is a chronic and progressive depletion of feline $CD4^+$ peripheral blood lymphocytes, a reduction in the CD4:CD8 cell ratio and, in some cases, an increase in CD8-bearing lymphocytes. Based on molecular, biochemical and immunopathologic characteristics, FIV infection of cats is now considered to be a better feline AIDS model than FeLV-FAIDS.

Cloning and sequence analysis of FIV has been reported in Olmsted *et al.* (1989a); Olmsted *et al.* (1989b); and Talbott *et al.* (1989). Hosie and Jarret (1990) described the serological response of cats infected with FIV. FIV virus subtypes can be classified according to immunotype based on the level of cross-neutralizing antibodies elicited by each strain (Murphy and Kingsbury, 1990). Recently, viruses have been classified into subtypes according to genotype based on nucleotide sequence homology. Although HIV and FIV subtyping is based on genotype (Sodora *et al.*, 1994; Rigby *et al.*, 1993; and Louwagie *et al.*, 1993), little is known about the correlation between the genotype and immunotype of subtypes. FIV viral isolates are currently classified into four FIV subtypes: A, B, C and D. (Kakinuma *et al.*, 1995). The following abbreviations of FIV strains are used herein:

Strain (subtype)	Abbreviation
Petaluma (A)	FIV_{Pet}
Dixon (A)	FIV_{Dix}
UK8 (A)	FIV_{UK8}
Bangston (B)	FIV_{Bang}
Aomori-1 (B)	FIV_{Aom1}
Aomori-2 (B)	FIV_{Aom2}
Shizuoka (D)	FIV_{Shi}

Infectious isolates and infectious molecular clones have been described for all FIV subtypes except for subtype C (Sodora *et al.*, 1994). Subtype C FIV has only been identified from cellular DNA of cats from Canada (Sodora *et al.*, 1994; Rigby *et al.*, 1993; Kakinuma *et al.*, 1995).

To date, there have been no reported cases of retroviral zoonosis between domestic cats and humans (Pedersen *et al.*, 1987; Yamamoto *et al.*, 1989; Yamamoto *et al.*, 1988; Butera *et al.*, 2000; CDC Report: HIV and Retrovirology). No cases of feline leukemia virus (FeLV), feline foamy virus (FeFV), and feline immunodeficiency virus (FIV) infections of humans have been reported, even in populations at high risk for viral exposure, such as veterinarians, animal caretakers, and scientists from feline retroviral laboratories (Yamamoto *et al.*, 1989; Yamamoto *et al.*, 1988; Butera *et al.*, 2000). Human patients with leukemia and chronic fatigue syndrome selected for their disease association also tested negative for FeLV (Butera *et al.*, 2000). However, many of the assays used in these studies were based on less sensitive antigen and antibody tests (Butera *et al.*, 2000). In few of these studies, a more sensitive FeLV PCR (polymerase chain reaction) system for proviral DNA and a sensitive Western blot analysis for FIV antibodies were performed but their findings also supported previous reports of the lack of feline retroviral zoonosis (Yamamoto *et al.*, 1988; Butera *et al.*, 2000). *In vitro* studies have shown that all three of these feline retroviruses are capable of infecting primary human cells and human cell (Butera *et al.*, 2000; Sarma *et al.*, 1970; Azocar *et al.*, 1979; Jarrett *et al.*, 1973). Recent studies have also demonstrated that FIV infects human cells *in vitro* via the CXCR4 receptor, which has been shown to be a coreceptor for HIV-1 (Willett *et al.*, 1997a; Willett *et al.*, 1997b; Poeschla *et al.*, 1998; Richardson *et al.*, 1999; Johnston *et al.*, 1999b). It has been reported that FIV vector sequences which included FIV *rev-RRE* and *gag* are more efficient in infecting human cells than those without FIV *gag* (Johnston *et al.*, 1999).

Zoonotic infection of humans with SIV has been limited to individuals working with SIV or SIV-infected laboratory animals (Khabbaz *et al.*, 1994; Khabbaz *et al.*, 1992). All of the SIV-infected individuals are clinically asymptomatic with one having transient infection while the other showed persistent infection.

Disclosed herein is the surprising discovery of zoonotic retroviral infection of humans *in vivo* with FIV. It has also been discovered that zoonotic FIV infection can complicate the

current HIV-1 antibody diagnostic tests that are used commercially to screen persons for infection with HIV.

Brief Summary of the Invention

The subject invention concerns materials and methods for detecting, preventing and treating retroviral infections in humans and other non-feline animals susceptible to infection by retrovirus. It has been discovered that FIV can be transmitted from cats to humans and that the FIV can infect human cells *in vivo*. Persons infected with FIV produce an immune response against the virus, including the production of antibodies to FIV. It has also been discovered that antibodies generated by a person infected with FIV cross-react with HIV antigens. Thus, the methods and compositions of the subject invention can be used to detect, prevent and treat FIV infection in humans and other non-feline animals that are susceptible to FIV infection. The present invention includes materials and methods for diagnosing whether a person is infected with FIV or HIV. The methods and compositions of the invention can also be used to prevent and treat infection by HIV in humans.

Brief Description of the Drawings

Figures 1A-1D show FIV Western blot analysis of subjects #FH1 and #FH2. FIV_{Shi} (D) and FIV_{Bang} (B) Western blots (Figures 1A-1C) were reacted with sera from subjects #FH1, #FH2, and #FH5 (control individual with minimum cat exposure) for 20 hours. Experimentally FIV-infected cat (Cat +) was used as the source of strongly reactive control serum and uninfected SPF cat (Cat -) was used as the source of non-reactive control serum. Key bands are highlighted with an arrowhead on the left. Figure 1D: Virus neutralizing antibodies to FIV and HIV were detected in cultures. a Western blot of human sera on FIV_{Shi}.

Figures 2A and 2B show alignment of gag sequences of cat #FC1 and subject #FH1. Figure 2A shows alignment of gag nucleotide sequences. Figure 2B shows alignment of gag amino acid sequences. Gag sequences of the nine clones isolated from cat #FC1 and subject #FH1 are shown in comparison to the consensus sequence of cat #FC1 (top sequence).

Hyphens denote nucleotide or amino acids identical to the consensus sequence derived from cat #FC1 and those, which differ from the consensus, are presented with the appropriate nucleotide or amino acid symbols.

Figures 3A-3C show HIV-1 Western blot analysis of subjects #FH1 and #FH2. Strongly reactive (++), weakly reactive (+), and non-reactive (-) control human sera from the Bio-Rad Novapath HIV-1 Immunoblot Kit and Cambridge Biotech HIV-1 Western Blot Kit were used as controls for respective HIV-1 Western blot strips. Serum from subject #FH5 with miminal exposure to cats, was used as additional negative control for Western blots from both companies. The durations of serum incubation are shown and FDA-approved recommended incubation periods are also designated with asterisk. Key bands are highlighted with an arrowhead on the left.

Figure 4 shows gag nucleotide sequence comparison of cat #FC1, subject #FH1 and FIV strains. Gag sequences of cat #FC1 and subject #FH1 were compared to all FIV strains available in our laboratory. The consensus sequence of subject #FH1 is shown at the top. Nucleotides identical to the consensus sequence of subject #FH1 (top sequence) are designated as a dot and those which differ from the consensus are presented with the appropriate nucleotide symbols. Gaps in sequence are presented as hyphens.

Figures 5A-5E show HIV-1 and FIV Western blot analysis of experimentally FIV-infected cats and pet cats. SPF cats #H3J, #D55, #455, and #X3D were experimentally infected with FIV_{Pet} (subtype A), FIV_{UK8} (subtype A), FIV_{Shi} (subtype D), and FIV_{Bang} (subtype A_{gag}/B_{Env}), respectively. FIV_{Bang} has Gag sequence of FIV subtype A and Env sequence of FIV subtype B. These serum were reacted with HIV-1 Western blots (Figures 5A and 5B) or FIV Western blots (Figures 5C, 5D, and 5E). Serum samples of these cats before FIV infection were negative by both FIV and HIV-1 Western blot analyses (data not shown). Serum from pet cats #FC1 and #FC2 were also tested for their reactivity to HIV-1 and FIV antigens. Cat #C9V (7 months post-inoculation serum shown) is an SPF cat inoculated with FIV isolated from pet Cat #FC1. All sera were incubated at serum dilution of 1:100. All procedures are identical to those described in Figures 1 and 3 unless stated

otherwise. Key bands are highlighted with an arrowhead on the left. FDA-approved serum incubation periods of 20 hours for Cambridge Biotech HIV-1 Western Blot Kit (Figure 5A) and 0.5 hour for Bio-Rad Novapath HIV-1 Immunoblot Kit (Figure 5B) were performed with the cat sera. Serum incubation for FIV Western blots was 20 hours (Figures 5C, 5D, and 5E).

Figures 6A-6C show HIV-1 and HTLV-1/2 immunoblot analysis of FIV-infected and FIV-vaccinated cat sera. Sera from FIV-infected cats and FIV-vaccinated cats were tested for cross-reactive antibodies to HIV-1 with BioRad Novapath HIV-1_{UCD1} and Cambridge Biotech HIV-1_{IIIB} immunoblot kits (Figures 6A & 6B) and to HTLV-1/2 with Cambridge Biotech HTLV-1/2 immunoblot kit (Figure 6C). Selected cat sera with unique banding patterns are shown to demonstrate the presence of cross-reactive antibodies with various patterns of reactivity to HIV-1 proteins. Serum samples of these cats before FIV inoculation were negative by both HIV-1 and HTLV-1/2 immunoblot analyses (data not shown).

Figures 7A and 7B show temporal development of cross-reactive antibodies to HIV-1. FIV and HIV-1 immunoblots are shown using selected sera from: Figure 7A, FIV-infected cats from different weeks post-inoculation (wk pi or pi); and Figure 7B, FIV-vaccinated cats from different weeks post-vaccination (post-vaccination number. Sera were compared to their pre-inoculation or pre-vaccination sera (Pre).

Figure 8 shows absorption of cat sera with viral antigens. Figure 8A: Cat sera were absorbed against inactivated FIV-infected cells followed by competition on HIV-1 immunoblots by inactivated FIV. Absorptions were also performed with PBS, uninfected cat FeT-J cells, and uninfected human H9/HuT-78 cells. Figure 8B: Sera were absorbed against PBS, uninfected cells lysate, or inactivated HIV-infected HuT-78 cells prior to incubation with HIV-1 immunoblot strips. Absorptions were performed for 2 hours at room temperature before development with anti-cat reagents. Figure 8C: FIV-vaccinated cat sera containing neutralizing antibodies to HIV-1 (Cat #C6G and #C9K) and sera from uninfected FeT-J cell immunized cats (Cats #C6E and #3G5) were tested at 1:100 dilution for reactivity to 5 μg/ml of either uninfected FeT-J cells, uninfected HuT-78 cells, or purified FIV_{Pet}.

Vaccinated cat sera had reactivity to FIV surface Env gp95 (arrow head). No significant reactivities were detected to uninfected FeT-J and HuT-78 proteins at 95 kDa, 120 kDa, and 160 kDa, suggesting that serum reactivity to HIV-1 and FIV envelopes were not due to nonspecific reactivity to cellular proteins. In addition, cats immunized with uninfected FeT-J cells had no reactivity to cellular proteins at 95 kDa but had antibodies reactive to cellular proteins close to 120 kDa and 160 kDa. However, these anti-cellular antibodies were close but distinctly different from reactivity to HIV-1 gp120 and gp160. Figure 8D: Serum from a cat immunized with uninfected FeT-J cells was absorbed against PBS, FeT-J, H9/HuT-78 cells, and FIV-infected Fet-J cells. Reactivities in serum from Cat #305 were readily absorbed against uninfected cat and human cells. Immunoglobulin levels of all absorbed sera were not significantly altered by infected-cell absorptions when compared to PBS and uninfected-cell absorbed sera. Seven % PAGE gels were used for developing immunoblots to increase resolution of high molecular weight proteins. Molecular weights (M) are presented in kDa.

Figures 9A and 9B show reactivity of FIV-vaccinated cat sera and PBMC to HIV p24 and gp160. Figure 9A: Sera from cats immunized with dual-subtype FIV vaccine were tested by ELISA using recombinant HIV-1_{BRU} p24, HIV-1_{IIIB} gp160, and FIV p24. ELISA results at serum dilution of 1:300 are presented as mean difference between pre- and post-vaccination sera. Figure 9B: PBMC from dual-subtype FIV vaccinated cats at 2 weeks post-5th vaccination were tested for interferon-γ production in response to recombinant HIV-1_{BRU} p24, HIV-1_{IIIB} gp160, and FIV p24. All PBMC stimulated with SEA were positive for IFNγ production (data not shown). The average of the triplicate samples are shown for IFNγ production. Standard deviations of the average IFNγ titer were less than 10% of the mean.

Figure 10 shows sequence alignments for partial FIV gag sequence from subject #FH1 PBMC following Real-time PCR.

Brief Description of the Sequences

SEQ ID NO. 1 is a sense primer or amplification of FIV *gag* that can be used according to the present invention.

SEQ ID NO. 2 is a antisense primer or amplification of FIV *gag* that can be used according to the present invention.

Detailed Description of the Invention

The subject invention concerns materials and methods for detecting, preventing and treating infection by FIV in humans and other non-feline animals susceptible to infection by FIV. The present invention is based on the surprising discovery that FIV can be transmitted from cats to humans and can infect human cells *in vivo*. Human subjects have been identified that are FIV positive and appear to have been infected through contact with their pet cats. Infection of humans by FIV has been demonstrated by confirmation of the presence of FIV nucleotide sequences in human cells using polymerase chain reaction (PCR) and by Western blot detection of FIV proteins expressed in human cells. Sequence analysis confirms that the subject is infected with FIV. Both of the human subjects infected with FIV identified thus far are currently clinically and immunologically asymptomatic. It has also been demonstrated that antibodies to FIV cross-react with HIV proteins. In addition, antibodies from FIV vaccinated animals can neutralize HIV-1 virus. The subject invention also concerns materials and methods for preventing and treating infection by HIV in humans.

One aspect of the subject invention concerns methods for detecting FIV infection of human cells. One method of the present invention comprises detecting the presence of antibodies that bind to an FIV protein or peptide, or nucleotide sequences of FIV. FIV diagnostic tests of the invention include ELISA, Western blot, and PCR tests. Current commercially available HIV antibody tests cross react with FIV proteins and, therefore, can give "false positive" results in subjects which are not infected with HIV but which are infected with FIV. Thus, FIV diagnostic tests for humans are needed in facilities doing HIV testing, such as hospitals and blood banks, in order to screen false positives and indeterminant results

obtained from current HIV tests. Methods for detecting and diagnosing FIV are known in the art and can be readily incorporated into assays for the testing of biological samples from humans for HIV infection. U.S. Patent Nos. 5,037,753, 5,118,602, 5,275,813, 5,510,106, and 5,565,319 describe assays and compositions for detecting FIV. Materials and methods for detecting and diagnosing HIV are disclosed in U.S. Patent Nos. 4,708,818, 5,055,391, 5,108,891, 5,135,684, and 5,922,533. Diagnostic HIV assays are also commercially available from Bio-Rad Laboratories, Hercules, CA. Methods of testing biological samples from humans for FIV infection only (and not HIV) are also contemplated by the present invention. Methods for FIV detection include PCR assaying for proviral FIV nucleotide sequences, RT-PCR assaying for FIV RNA nucleotide sequences, oligonucleotide probe assays (including Real-time PCR), and antibody-based assays. Antibody-based assays include, for example, methods to detect the presence of antibodies to FIV, such as ELISA and Western blots, and methods to detect the presence and/or expression of FIV proteins in human biological samples. In one embodiment, a biological sample from a human that is being assayed for the presence of antibodies to HIV or HIV sequences is assayed for the presence of antibodies to FIV or FIV sequences.

The present invention concerns materials and methods for inducing an immune response to FIV in a human or non-feline animal that is susceptible to infection by FIV. The present invention also concerns materials and methods for inducing an immune response to HIV in a human. In one embodiment, an amount of an FIV immunogen effective to induce an immune response is administered to the human or animal. FIV immunogens that can be used include, for example, synthetic FIV peptide, natural or recombinant FIV protein or a fragment thereof, polynucleotide comprising a sequence that encodes an FIV protein or fragment thereof, polynucleotide comprising a sequence that encodes an FIV protein or a fragment thereof and an HIV protein (such as NeF protein) or a fragment thereof, inactivated or attenuated whole FIV viral isolate, FIV viral fragment, inactivated cells infected with FIV, and compositions comprising FIV and HIV proteins or fragments thereof.

The present invention also concerns materials and methods for preventing FIV infection in humans and other animals. Specifically contemplated are methods and vaccine compositions which can be administered to human subjects and other susceptible host animals which will prevent infection by FIV. In one embodiment, an amount of an FIV immunogen effective to induce an immune response is administered to the human or animal. FIV immunogens that can be used include, for example, synthetic FIV peptide, natural or recombinant FIV protein or a fragment thereof, polynucleotide comprising a sequence that encodes an FIV protein or fragment thereof, polynucleotide comprising a sequence that encodes an FIV protein or a fragment thereof and an HIV protein (such as NeF protein) or a fragment thereof, inactivated or attenuated whole FIV viral isolate, FIV viral fragment, inactivated cells infected with FIV, and compositions comprising FIV and HIV proteins or fragments thereof. In a preferred embodiment, the FIV immunogen comprises an epitope of an FIV protein, such as core gag protein or envelope protein, that is evolutionarily conserved between FIV and HIV. Persons that have higher exposure to cats, such as veterinarians, scientists that use cats for research purposes, cat breeders, etc., would be candidates for receiving a vaccine treatment. Other animals which can be treated according to methods of present invention include dogs, horses, and captive non-domesticated animals such as those found in zoos and circuses, including tigers and lions.

The subject invention also concerns materials and methods for treating persons and other animals that are infected with FIV. In one embodiment, an effective amount of a composition which can induce an immune response against FIV is administered to a person or animal in need of such treatment. In another embodiment, one or more antiretroviral drugs can be administered to the person or animal. Antiretroviral drugs which can be used in the present invention include, but are not limited to, nucleoside analogs, such as azidothymidine (AZT) and lamivudine (3TC), non-nucleoside inhibitors of retroviral reverse transcriptase, and retroviral protease inhibitors. Published international patent application WO 99/60988 describes the use of a combination of AZT, 3TC, and a retroviral protease inhibitor to treat

FIV infection. In a further embodiment, polynucleotide sequences that are antisense to nucleotide sequences of FIV can be administered to the person or animal.

In another embodiment, a person or animal can be treated using one or more antibody that cross-reacts with both FIV and HIV antigens. Alternatively, a cocktail of one or more antibody that is specific to an FIV antigen and one or more antibody that is specific to an HIV antigen can be administered.

In another embodiment, a person or non-feline animal can be treated using an FIV immunogen that induces an immune response against FIV. FIV immunogens that can be used include, for example, synthetic FIV peptide, natural or recombinant FIV protein or a fragment thereof, polynucleotide comprising a sequence that encodes an FIV protein or fragment thereof, polynucleotide comprising a sequence that encodes an FIV protein or a fragment thereof and an HIV protein (such as NeF protein) or a fragment thereof, inactivated or attenuated whole FIV viral isolate, FIV viral fragment, inactivated cells infected with FIV, and composition comprising FIV and HIV proteins or fragments thereof.

In a further embodiment, antiretroviral drugs can be used in combination with FIV immunogen treatment or antibody therapy, or both, described above. Other animals which can be treated according to methods of present invention include dogs, horses, and captive non-domesticated animals such as those found in zoos and circuses, including tigers and lions.

The subject invention also concerns materials and methods for preventing HIV infection in humans that are not infected with HIV. In one embodiment, an FIV immunogen is administered to a person. In a preferred embodiment, the FIV immunogen induces an immune response against one or more subtypes of FIV. FIV immunogens that can be used include, for example, synthetic FIV peptide, natural or recombinant FIV protein or a fragment thereof, polynucleotide comprising a sequence that encodes an FIV protein or fragment thereof, polynucleotide comprising a sequence that encodes an FIV protein or a fragment thereof and an HIV protein (such as NeF protein) or a fragment thereof, inactivated or attenuated whole FIV viral isolate, FIV viral fragment, inactivated cells infected with FIV, and composition comprising FIV and HIV proteins or fragments thereof. In another

embodiment, a person or animal can be administered an FIV immunogen and then subsequently receive a secondary administration of an HIV immunogen. Preferably, the FIV immunogen elicits an immune response against more than one FIV subtype. HIV immunogens can include core gag protein (p24) and envelope protein (gp100/gp120). In a preferred embodiment, the FIV immunogen comprises an epitope of an FIV protein, such as core gag protein or envelope protein, that is evolutionarily conserved between FIV and HIV.

The invention also concerns materials and methods for treating persons that are already infected with HIV. In one embodiment, an FIV immunogen is administered to a person. In a preferred embodiment, the FIV immunogen induces an immune response against one or more subtypes of FIV. FIV immunogens that can be used include, for example, synthetic FIV peptide, natural or recombinant FIV protein or a fragment thereof, polynucleotide comprising a sequence that encodes an FIV protein or fragment thereof, polynucleotide comprising a sequence that encodes an FIV protein or a fragment thereof and an HIV protein (such as NeF protein) or a fragment thereof, inactivated or attenuated whole FIV viral isolate, FIV viral fragment, inactivated cells infected with FIV, and composition comprising FIV and HIV proteins or fragments thereof. In another embodiment, a person or animal can be administered an FIV immunogen and then subsequently receive a secondary administration of an HIV immunogen. Preferably, the FIV immunogen elicits an immune response against more than one FIV subtype. HIV immunogens can include core gag protein (p24) and envelope protein (gp100/gp120).

In another embodiment, a person or animal infected with HIV can be treated using one or more antibody that cross-reacts with both an FIV protein or antigen and an HIV protein or antigen. Alternatively, a cocktail of one or more antibody that is specific to an FIV antigen and one or more antibody that is specific to an HIV antigen can be administered.

In a further embodiment, antiretroviral drugs can be used in combination with FIV immunogen treatment or antibody therapy, or both, described above. Antiretroviral drugs for treating HIV are known in the art and include nucleoside analogs such as azidothymidine

(AZT) and lamivudine (3TC), non-nucleoside inhibitors of retroviral reverse transcriptase, and retroviral protease inhibitors.

The compositions of the invention, when administered to a human or other animals susceptible to FIV infection, can induce protective humoral and/or cellular immune responses against infection by FIV. Preferably, the composition can induce immune responses against multiple strains of FIV. More preferably, the compositions of the invention can induce immune responses against homologous and heterologous strains of FIV. The compositions can be, for example, composed of synthetic FIV peptide, natural or recombinant FIV protein or a fragment thereof, polynucleotide comprising a sequence that encodes an FIV protein or fragment thereof, polynucleotide comprising a sequence that encodes an FIV protein or a fragment thereof and an HIV protein (such as NeF protein) or a fragment thereof, inactivated or attenuated whole FIV viral isolate, FIV viral fragment, inactivated cells infected with FIV, and composition comprising FIV and HIV proteins or fragments thereof, or a combination of any of the above. In a preferred embodiment, the vaccine composition of the subject invention comprises peptides, proteins or strains of FIV from two different FIV subtypes. In one embodiment, FIV subtype A and FIV subtype D are represented in the composition. Preferably, the composition comprises peptides, proteins or viral isolates from three FIV strains, each strain from a different FIV subtype. More preferably, at least one FIV strain from each of FIV subtype A, subtype B and subtype D is included in the vaccine composition. Compositions directed to multiple subtypes of FIV are described in U.S. Patent No. 5,846,825.

The compositions of the subject invention also encompass recombinant viral vector-based FIV constructs that may comprise, for example, FIV *env*, *gag/pro*, or *env-gag/pro*. Any suitable viral vector that can be used to prepare recombinant vector/FIV constructs is contemplated for use with the subject invention. For example, viral vectors derived from adenovirus, avipox, feline herpesvirus, vaccinia, canarypox, entomopox, swinepox and others known in the art can be used with the compositions and methods of the present invention. Recombinant polynucleotide vectors that encode and express FIV

components can be constructed using standard genetic engineering techniques known in the art. In addition, the various compositions described herein can be used separately and in combination with each other. For example, primary immunizations of a person or other animal may utilize recombinant vector-based FIV constructs, having single or multiple subtype components, followed by secondary boosts with vaccine compositions comprising synthetic FIV peptides and/or recombinant FIV proteins. Other immunization protocols with the vaccine compositions of the invention are apparent to persons skilled in the art and are contemplated within the scope of the present invention.

Natural, recombinant or synthetic polypeptides of FIV viral proteins, and peptide fragments thereof, can also be used as compositions according to the subject methods. In a preferred embodiment, FIV polypeptides derived from multiple FIV subtypes are combined in a vaccine composition and are used to vaccinate a human or other susceptible animal in need of such treatment. For example, polypeptides based on the FIV envelope glycoprotein from at least two prototype FIV strains from different subtypes can be combined in the vaccine. The polypeptides may be homologous to one strain or may comprise "hybrid" or "chimeric" polypeptides whose amino acid sequence is derived from joining or linking polypeptides from at least two distinct FIV subtypes. Procedures for preparing FIV polypeptides are well known in the art. For example, FIV polypeptides can be synthesized using solid-phase synthesis methods (Merrifield, 1963). FIV polypeptides can also be produced using recombinant DNA techniques wherein a polynucleotide molecule encoding an FIV protein or peptide is expressed in a host cell, such as bacteria, yeast, or mammalian cell lines, and the expressed protein purified using standard techniques of the art.

According to the methods of the subject invention, the FIV vaccine compositions described herein are administered to a human or other animal susceptible to FIV infection in an effective amount and in a manner capable of inducing protective immunity against subsequent challenge or infection of the human or animal by FIV. The vaccines are typically administered parenterally, by injection, for example, either subcutaneously, intraperitoneally, or intramuscularly. Other modes of vaccine administration contemplated by the invention

include oral or nasal administration. Usually, the vaccines are administered to a subject at least two times, with an interval of one or more weeks between each administration. However, other regimens for the initial and booster administrations of the vaccine are contemplated, and may depend on the judgment of the practitioner and the particular host animal being treated.

The vaccine compositions of the subject invention can be prepared by procedures well known in the art. For example, the vaccines are typically prepared as injectables, *e.g.*, liquid solutions or suspensions. The vaccines are administered in a manner that is compatible with dosage formulation, and in such amount as will be therapeutically effective and immunogenic in the recipient. The optimal dosages and administration patterns for a particular vaccine formulation can be readily determined by a person skilled in the art.

Virus for use in a vaccine formulation may be inactivated or attenuated using methods known in the art. For example, whole virus and infected cells can be inactivated or attenuated by exposure to paraformaldehyde, formalin, phenol, UV light, elevated temperature and the like.

In one embodiment, a biological sample, such as blood, serum, saliva and the like, is obtained from a person and assayed for the presence of antibodies that can bind specifically to FIV or an FIV antigen. The sample can be assayed for antibodies that bind with all subtypes or strains of FIV or antigens thereof, as well as for antibodies that are specific to a particular subtype or strain of FIV or antigen thereof so as to facilitate diagnosis of the FIV subtype or strain infecting the subject. The sample can optionally be assayed for the presence of antibodies that bind to HIV or an HIV antigen. Assay techniques, *e.g.*, ELISA and Western blotting, for detecting antibodies to FIV and HIV are known in the art. In another embodiment, a biological sample is assayed for the presence of FIV-specific nucleotide sequences and/or proteins. Standard PCR and nucleotide hybridization techniques can be used to amplify and detect the presence of FIV-specific nucleotide sequences in a sample. RT-PCR can be used to detect FIV RNA sequences. FIV oligonucleotide primers and probes for use in such techniques and which are substantially complementary with a portion of the

FIV genomic sequence or FIV RNA sequences can be readily prepared based on known FIV sequences.

Polymerase chain reaction (PCR) is based on repeated cycles of denaturation of double-stranded DNA, followed by oligonucleotide primer annealing to the DNA template, and primer extension by a DNA polymerase (Mullis *et al.* Patent Nos. 4,683,195, 4,683,202, and 4,800,159; Saiki *et al.* 1985). The oligonucleotide primers used in PCR are designed to anneal to opposite strands of the DNA, and are positioned so that the DNA polymerase-catalyzed extension product of one primer can serve as the template strand for the other primer. The PCR amplification process results in the exponential increase of discrete DNA fragments whose length is defined by the 5' ends of the oligonucleotide primers. Nucleotide hybridization methods are disclosed, for example, in U.S. Patent No. 4,358,535.

The subject invention also concerns antibodies that cross-react with both FIV and HIV antigens. Antibodies can be prepared and isolated using standard methods known in the art. For example, a suitable animal can be immunized with with an FIV immunogen via one or more intramuscular or subcutaneous injections, optionally with an adjuvant, over a period of time. Immunized animals can be periodically bled and antibodies isolated from the antisera. The anti FIV antibodies can then be screened for cross-reactivity with FIV. Antibodies of the present invention can be polyclonal or monoclonal. Monoclonal antibodies can be prepared according to the methods of Kohler and Milstein (1976). In those cases where a monoclonal antibody of the present invention is to be used in a human, the antibody can be "humanized" to minimize immune reactions against the antibody by the human system. Techniques for humanizing antibodies are well known in the art and are described in U.S. Patent No. 5,807,715, 5,693,762, 5,585,089, 5,530,101, and Morrison *et al.* (1984).

The subject invention also concerns compositions comprising at least one FIV protein and/or antigen or fragment thereof and at least one HIV protein and/or antigen or fragment thereof. In one embodiment, the composition comprises epitopes of FIV and HIV proteins that are evolutionarily conserved between the viruses. In a preferred embodiment, the composition comprises core gag protein and/or viral envelope protein.

The subject invention also concerns polynucleotide molecules that encode at least one FIV protein or fragment thereof and at least one HIV protein (such as NeF) or fragment thereof.

The subject invention also concerns materials and methods for preventing infection in humans and other animals by lentivirus such as CAEV and SIV.

All patents, patent applications, provisional applications and publications referred to or cited herein are incorporated by reference in their entirety to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 – Immunization of Humans with FIV

Western Blot Analysis: FIV-Petaluma (FIV_{Pet}, subtype A), FIV-Shizuoka (FIV_{Shi}, subtype B) and FIV-Bangston (FIV_{Bang}, subtype D) Western blots were reacted with sera from subjects #FH1, #FH2, and #FH5 and from cats #FC1, #FC2, and #C9V at 1:100 dilution for 20 hours (room temperature). Subject #FH5 served as a control individual, having had minimal exposure to cats. Experimentally FIV-infected cats were used as the source of strongly reactive control sera. Uninfected specific pathogen-free (SPF) cats were used as the source of non-reactive control sera. Except for the FIV mini-Western blot strips and the duration of incubation, all reagents and methods for testing human sera were identical to those described in Bio-Rad Novapath HIV-1 Immunoblot Kit (Bio-Rad Laboratories, Hercules, CA). FIV mini-Western blot strips were produced using sucrose-gradient purified FIV at 1.5 μg/strip as previously described (Yamamoto *et al.*, 1988a). Cat control sera were tested using the alkaline phosphate enzyme-linked anti-cat IgG antibodies (Chemicon International Inc., Temecula, CA) at 0.12 μg/ml and all procedures were performed in a total

volume of 1.5 ml per strip. Aside from these modifications, the reagents and procedure for testing cat sera were identical to those described in the Bio-Rad Western blot kit.

Positive and negative control human sera from the Bio-Rad HIV-1 Immunoblot Kit and Cambridge HIV-1 Western Blot Kit (Cambridge Biotech, Rockville, MD) were used as controls for the respective HIV-1 Western blot strips. Serum from subject #FH5 was used as an additional negative control for Western blots from both companies. Except for the duration of incubation, all reagents and methods for testing human sera were identical to those described in Bio-Rad's and Cambridge's HIV-1 Western blot kits. Serum from FIV-infected cats collected from 6 to 32 months post-infection were also tested with HIV-1 or FIV Western blots.

MolecularAnalysis: Primary PBMC were cocultured with mitogen-stimulated PBMC from either a FIV-seronegative individual or an SPF cat. The mitogens used were Staphylococcal enterotoxin A (SEA, 0.5 μg/ml) or concanavalin A (Con A, 1 μg/ml). After 1.5 to 3 weeks of culture, RNA and genomic DNA were extracted from the cultured PBMCs by guanidine isothiocyanate-phenol procedure using TRIZOL Reagent (GIBCO BRL, Rockville, MD) and QIAamp DNA blood Mini kit (QIAGEN, Valencia, CA), respectively. The levels of reverse transcriptase (RT) activity present in cultured fluids were measured by a RT assay (Yamamoto *et al.*, 1988b). Viral RNA was extracted from plasma using QIAamp Viral RNA Mini kit (QIAGEN, Valencia, CA). After DNase treatment, RNA was reverse transcribed to cDNA with AMV RT and random hexamer primers. One μg of cDNA from cell associated RNA, cDNA from plasma RNA (in 62.5μl of plasma), and genomic DNA from $1x10^5$ cells were used for PCR analysis. Nested PCR for FIV *gag* was performed using primer sets as previously described (Hohdatsu *et al.* 1998). FIV whole gag genes were amplified by single PCR with sense primer GAGF (5'-CAACAAGGTAGGAGAGATTCTACA-3') (SEQ ID NO:1) and antisense primer, GAGR (5'-TAAAATTGTTATATCTGCTCCTGT-'3) (SEQ ID NO:2). Primers used for detection of HIV-1 were previously described (Ou *et al.*, 1988). Amplification products of the expected size were cloned into the pCR 2.1 TOPO cloning vector (Invitrogen, Carlsbad,

CA) and sequenced (ICBR core lab, UF). Sequences of FIV strains and other retroviruses were obtained from NCBI/GenBank and analyzed by CRYSTAL W program and Basic Local Alignment Search Tool (BLAST).

Whole blood from subject #FH1 was submitted to College of Veterinary Medicine at the University of Florida by the physician upon the subject's request. Positive antibody reactivity was found by Western blot to FIV major core capsid p24 (Ca) protein and precursor Gag p55, with weak reactivity to FIV minor core nucleoprotein p10 and potential FIV RT p65 protein (Figures 1A-D). The reactivities observed on these blots are not likely to be non-specific, since reactivity to p24 was abrogated by preasborption of sera with FIV-infected cells (data not shown). Sera from #FH1 and #FH2 were able to neutralize FIV, but not HIV-1, *in vitro*. Antibody reactivity of #FH1 to FIV proteins could have been induced by antigenic stimulation via proteins shed by the subject's FIV infected cat or could be the result of actual infection. Hence, PBMC and plasma from #FH1 were tested for FIV. Plasma was negative for FIV *gag* by nested RT-PCR and cells were negative by FIV proviral PCR. To determine the presence of infected cells, a sensitive coculture amplification technique was used in which PBMC from the subject were cocultured with concanvalin A (Con A)-stimulated PBMC from either a FIV-seronegative individual or a SPF laboratory cat. Only a human-human cocultured cell preparation was positive for a 300 bp sequence at the p24 region by nested RT-PCR. RT-PCR (without nesting) with FIV *gag* primers determined the presence of a 1500 bp band indicating detection of FIV *gag* RNA (Figures 2A-B). The control culture consisting of cells from SPF cat or FIV-seronegative individual were negative by the same assays. Thus, the entire *gag* gene was found to reside in PBMC from the #FH1 subject. Culture fluids were negative for FIV by RT assay.

Since a full RNA *gag* sequence was derived from PBMC, serum from #FH1 was retested using FDA-approved HIV-1Western blot assays (Figures 3A-C). The previous HIV-1 Western blot test performed by licensed diagnostic laboratories used the Western blot kit from Cambridge (HIV-1_{IIIB}, subtype B). Western blot strips from Bio-Rad Laboratories use HIV-1_{UCD1} (subtype B) as the source of viral proteins. Serum from #FH1 reacted to both

sets of strips at p24 and weakly at p55, but unlike FIV Western blot results, no minor core and RT reactions were observed. This pattern of serum reactivities to HIV and FIV proteins suggest that the subject's antibodies were more likely induced by infection with FIV rather than HIV-1 (Figures 1A-D and 3A-C). Further, reactivities to HIV-1 p24 on both HIV-1_{IIIB} and HIV-1_{UCD1} strips, HIV-1 p55 only on HIV-1_{UCD1}, and FIV p10, p24, and p55 proteins, suggest that antibodies from #FH1 were reacting to evolutionarily retained epitopes on precursor Gag proteins and products. Antibody reactivities that cross lentiviral species have been reported (Matsuo *et al.*, 1992; Olmsted *et al.*, 1989a). Polyclonal rabbit antibodies to ruminant lentiviruses (caprine arthritis encephalitis virus, CAEV, and visna virus, VV) react to FIV major (p24) and minor (p10/17) core proteins on Western blot (Olmsted *et al.*, 1989a). However, sera from FIV-infected cats have been reported to lack cross-reactive antibodies to HIV-1 and visa versa (Pedersen *et al.*, 1987; Yamamoto *et al.*, 1988b).

Upon determination of positive FIV serology and positive FIV *gag* nested RT-PCR of the subject #FH1, the source of the FIV infection was investigated. FIV was isolated and sequenced from the FIV-seropositive pet cat #FC1. FIV from cat #FC1 was readily isolated from PBMC by coculturing with Con-A-stimulated PBMC from an SPF cat, and the culture fluids were positive for FIV by RT assay. The cocultured cells were positive for FIV *gag* by both proviral PCR and RT-PCR. Control cultures consisting of cells from a SPF cat were negative by the same assays. Nested RT-PCR of plasma from Cat #FC1 also tested positive for FIV RNA *gag*. The ease in isolating FIV from PBMC and plasma suggests that the viral load in this cat was higher than subject #FH1. A comparison of the full *gag* sequences from subject #FH1's human-human cocultured cells and her cat's cat-cat cocultured cells revealed 99.6% nucleotide and 99.3% amino acid sequence homologies, demonstrating that this individual most likely contracted the FIV infection from her pet cat #FC1 (Figures 2A-B). Based on sequence analysis at Gag/*gag*, sequence from Cat #FC1 belonged to FIV subtype B. Gag/*gag* sequences from Cat #FC1 had 83.1-94.2% nucleotide and 87.4-98.2% amino acid homology to subtype B FIV isolates ($FIV_{Aomori-1}$, $FIV_{Aomori-2}$, $FIV_{Sendai-2}$, $FIV_{Yokohama}$, FIV_{TM2}) but were clearly different from the FIV strains (<85.2% nucleotide and <88.6% amino acid

homology) which were being produced in our laboratory (Figure 4, amino acid sequences shown). Thus, the sequences derived from subject #FH1 and her cat, were not due to contamination from laboratory strains of FIV. These sequences had <55% nucleotide and amino acid sequence homologies to HIV-1, HIV-2, HTLV, SIV, EIAV, CAEV, VV, FeLV, and FeFV and were clearly distinct from the primate, ungulate, and other feline retroviruses (data not shown). Furthermore, BLAST analysis against NCBI/GenBank indicated that no known human protein sequences had any significant degree of homology to FIV Gag protein.

Since subject #FH2 has previously worked with HIV-1, she was tested for HIV-1 infection by PCR and HIV-1 antibodies by commercial HIV-1 Western blot analysis upon request by the subject. Based on standard HIV-1 Western blot analyses using Cambridge Biotech (20-hour serum incubation) and Bio-Rad Laboratories (30-minute serum incubation) tests, this subject was negative for HIV-1 antibodies. This finding was also confirmed by a licensed diagnostic laboratory. However, upon longer incubation period (20 hours) on Bio-Rad Western blot strip, faint antibody reactivity to p24 was observed repeatedly using serum collected from subject #FH2 on two different days in 2001 but slightly stronger reactivity to p24 was detected in serum collected after 1993. Both direct and coculture amplified PBMC from subject #FH2 were negative for HIV-1 by both PCR and RT-PCR with HIV-1 p24 *ca* primers. This result was confirmed by a licensed diagnostic laboratory using RT-PCR (Roche Amplicore HIV-1 Monitor Test). Sera from #FH2 were next tested for the presence of antibodies to FIV proteins. The more recent sera were strongly positive for antibodies reactive to FIV p10, p24, and p65 (potential FIV RT protein) and weakly positive for antibodies reactive to FIV p55 by Western blot analysis (Figures 1A-D). Her sera from 1993, collected before her participation in HIV research, reacted weakly to p55 of FIV-Petaluma (FIV_{Pet}), FIV-Shizuoka (FIV_{Shi}) and FIV-Bangston (FIV_{Bang}) and reacted strongly to all FIV p24 and p10. This observation together with our Gag/*gag* sequence results suggests that this subject is actively or defectively infected with FIV. In order to determine the source of FIV infection, her pet cat #FC2 was tested for FIV. Cat #FC2 was negative for FIV infection by RT and PCR and for FIV antibodies by Western blot analysis

suggesting that her previous pet cat was more likely the source of FIV infection. This observation further indicates that subject #FH2 has been infected with FIV for at least 3 years since her previous pet cat died 3 years ago and possibly as long as 7.5 years since her serum from 1993 also reacted to FIV proteins and to HIV-1 p24 and p55.

To determine if sera from FIV-infected cats can cross-react with HIV-1, HIV-1 Western blot analyses were performed on sera from SPF cats experimentally infected for prolonged period of time with four FIV strains currently being produced in our laboratory (Figures 5A and 5B). Sera from pet cats #FC1 and #FC2 were also tested along with sera from a SPF cat and a SPF cat (#C9V) infected with FIV_{FC1} isolated from pet Cat #FC1. Sera from cats infected with either FIV_{Pet} (cat #H3J) or FIV_{UK8} (cat #D55) reacted strongly to HIV-1 p24 or p32 (HIV-1 integrase), respectively on both Bio-Rad HIV-1_{UCD1} and Cambridge Biotech HIV-1_{IIIB} Western blots. In addition, serum from FIV_{UK8}-infected cat #D55 reacted weakly to p24 on Cambridge Biotech HIV-1_{IIIB} strip. Sera from Cat #FC1 reacted weakly to p24 of both HIV-1_{IIIB} and HIV-1_{UCD1}. However, cat #C9V infected with FIV_{FC1} was strongly reactive to p24 of both HIV-1 strains. Hence, FIV_{FC1} core sequence which has 99.3% amino acid homology to FIV sequence isolated from subject #FH1, can readily induce cross-reactive antibodies to HIV-1. In contrast, sera from cat #FC2 and cats infected with FIV_{Shi} (cat #455) and FIV_{Bang} (cat #X3D) were non-reactive to HIV-1 proteins on both HIV-1 Western blots. All FIV-infected laboratory cats and cat #FC1 were strongly positive for FIV antibodies (Figures 5C and 5D). Although the numbers of serum samples are small, it is interesting to note that not all long-term FIV-infected cats produced anti-FIV antibodies that cross-reacted with HIV-1 p24 and p32. Nevertheless, these initial results indicate that sera from certain FIV-infected cats as well as a human subject positive by nested RT-PCR for FIV p24 *ca* or *gag* sequences do react to HIV-1 p24 protein. Thus, infection with FIV in humans results in antibodies that cross-reacts to HIV-1 p24 and HIV-1 p32.

Example 2 – FIV Antigens Induce Cross-reactive Immunity to HIV-1

Vaccinated and Infected Animals: Specific-pathogen-free cats were obtained from the investigator's SPF breeding colony or purchased from Liberty Research (Waverly, New York) and Cedar River Laboratories (Mason City, Iowa). All SPF cats tested negative for toxoplasma, feline leukemia virus, and FIV before experimental infection. These cats were immunized at 2-6 weeks intervals with FIV_{Pet} and FIV_{Shi} vaccines at combined or single dose of 250-500 μg for whole virus vaccine and 2.5-5x10^6 cells for inactivated infected-cell vaccines and sera collected at 2-4 weeks post vaccination (Pu *et al.*, 2001). SPF cats were inoculated intravenously with 10-100 median cat infectious doses (CID_{50}) of either in vitro-derived or in vivo-derived inoculum as previously described (Pu *et al.*, 2001).

Immunoblot, ELISA, and VN Antibody Analyses: Commercial HIV-1 (BioRad, Hercules, California; Cambridge Biotech, Rockville, Maryland) and HTLV-I and II (Cambridge Biotech) immunoblot strips were performed at 1:100 serum dilution using the methods described in the kits except for the anti-cat reagents and for the absorption/competition studies. Alkaline-phosphatase conjugated goat anti-cat IgG (Chemicon, Temecula, California) at 0.3μ g/ml and biotinlylated anti-cat IgG (Vector, Burlingame, California) at 0.4 μg/ml were used in place of the anti-human reagents for testing feline serum. ELISA assays were developed using commercial recombinant HIV-1_{IIIB} gp160 (Chemicon), HIV-1_{BRU} p24 (Biodesign, Kennebunk, Maine), and FIV p24 (Fort Dodge, Fort Dodge, Iowa) using a method as previously described (Yamamoto *et al.*, 1993) with the following modifications. HIV-1 p24 and gp160 were coated on the plate at 300 and 150 ng/well, respectively. FIV 24 was coated at 50 ng/well. All samples for ELISA were performed in triplicates. The HIV VN antibody assay will be identical to the FIV VN antibody assay with the exception of SEA-stimulated human PBMC as indicator cells and HIV-1_{LAV} (20 $TCID_{50}$) or HIV-1_{UCD1} (0.1-50 $TCID_{50}$) as inocula (Pu *et al.*, 2001; Yamamoto *et al.*, 1993).

Absorption and Competition with Viral Antigens: Cat sera were absorbed three times for 1 hr each with either 2x10^8 infected or uninfected cells followed by competition with 250

μg of UV-inactivated virus or cell lysate directly on the immunoblot strips with the serum for 2 hr and the immunoblots were developed as before. FIV-infected (FIV_{Shi}-infected FeT-J and FIV_{Bang}-infected FeT-J cell combination), HIV-infected (HIV-1_{UCD1} infected HuT-78 and HIV-1_{LAV} infected H9 cell combination) and uninfected (FeT-J alone or HuT-78/H9 combination) cells were inactivated by 0.6% paraformaldehyde. HIV-infected cells were also UV-inactivated before paraformaldehyde treatment. IgG levels of the cell-absorbed and unabsorbed mock sera were determined by commercial feline IgG radial-immunodiffusion assay (Bethyl Laboratory, Montgomery, Texas).

Cellular Immune Response: Virus-specific cellular immune responses of PBMC from vaccinated cats were determined by measuring the amount of interferon-γ produced in response to 10 μg/ml of recombinant FIV p24, HIV-1_{BRU} p24, and HIV-1_{IIIB} gp160 using the method previously described (Pu *et al.*, 1999). In addition, cells stimulated with uninfected cell lysate (20 μg/ml), SEA (0.2 μg/ml, positive control), media diluent (negative control), and purified whole FIV_{Pet} and FIV_{Shi} (20 μg/ml) were also included as additional controls.

Antibodies to FIV were developed in specific pathogen free (SPF) cats by either active infection with FIV strains or immunization with inactivated FIV vaccines. Sera from 41 FIV-infected cats at different time post-FIV inoculation were evaluated on BioRad HIV-1_{UCD1} and Cambridge Biotech HIV-1_{IIIB} immunoblots (Table 1, Figure 6A). Overall, 18 of 41 (44%) infected cats had antibodies to HIV-1 core capsid p24, matrix p18, Gag p55, intergrase p32, transmembrane envelope gp41, surface envelope gp120 or precursor envelope gp160 (Table 1, Figure 6A) with greatest reactivity to p24. Three of 10 cats infected with FIV_{Pet} (subtype A), 7 of 11 cats infected with FIV_{UK8} (subtype A), 5 of 11 cats infected with FIV_{Bang} (subtype A_{gag}/B_{env}), and 3 of 9 cats infected with FIV_{Shi} (subtype D) had cross-reactive antibodies to HIV-1. The majority of the cats (64%) infected with FIV_{UK8} developed cross-reactive antibodies to HIV-1, while only three cats (30%) infected with FIV_{Pet} developed cross-reactive antibodies to HIV-1. Both of these strains are subtype A FIV strains. Hence, strain specific cross-reactivity to HIV-1 may exist.

Similarly, sera from FIV-vaccinated cats were tested on BioRad HIV-1_{UCD1} and Cambridge HIV-1_{IIIB} immunoblots (Table 1, Figure 6B). The vaccinated cat sera had greater reactivity and recognized more HIV-1 proteins than did sera from infected cats. Sera that reacted with HIV-1_{IIIB} p24 consistently reacted to HIV-1_{UCD1} p24 but not vice versa. Cross-reactivity to HIV-1 envelope products was observed mostly with HIV-1_{UCD1} rather than HIV-1_{IIIB}. In contrast, sera reactive to HIV-1 polymerases p51 and p66 reacted to the viral polymerases from only HIV-1_{IIIB}. HIV-1_{IIIB} and HIV-1_{UCD1} are subtype B isolates that were produced in human H9 and HuT-78 cells, respectively. HIV-1_{IIIB} and HIV-1_{UCD1} has 97% amino acid sequence homology at p24 and 85% amino acid sequence homology at Env. Thus, the specificity of the cross-reactivity appears to differ based on the HIV-1 strain at polymerases and Env. Six FIV-infected and six FIV-vaccinated cats that reacted to HIV-1 immunoblot were tested against HTLV-I/II using Cambridge HTLV-I/II Western Blot Kit. Three vaccinated and two infected cats had cross-reactive antibodies to HTLV-I/II core p24 (Figure 6C). One of the infected cat also had antibodies weakly reactive to HTLV Tax p38, while the other infected cat also had antibodies weakly reactive to HTLV precursor Gag p53 and Gag intermediate p42. As previously reported for lentiviruses (Olmsted *et al.*, 1989a; Matsuo *et al.* 1992; Goudsmit *et al.*, 1986), core sequences appear to be evolutionarily conserved even between retrovirus families (Egberink *et al.*, 1991).

Since the post-infection sera that were tested were not necessarily collected at the optimal time for the presence of cross-reactivity antibodies to HIV-1, a study was performed using cat sera from different times post FIV infection (pi) (Figure 7A). Cross-reactive antibodies to HIV-1 were detected in sera from all five cats at relatively early stage of FIV infection (10-14 wk pi), at which time only 1-2 bands developed (predominantly HIV-1 p24 protein), and persisted for a long-time. Comparing HIV and FIV immunoblots with sera from the same time points, antibodies that recognized FIV proteins developed earlier (2-6 wk pi) and reacted persistently at higher titers and to a broad spectrum of FIV proteins (Figure 7A). Furthermore, FIV antibodies from infected cats that were negative for HIV-1 cross-reactivity were negative throughout 6-120 wk pi (data not shown). The temporal development of the

cross-reactive antibodies to HIV-1 in FIV vaccine sera was next determined (Figure 7B). The cross-reactive antibodies developed as early as second vaccination and persisted for a prolonged period of time. Similar to anti-p24 antibodies in the infected cat sera, the antibodies reactive to FIV p24 developed much earlier than those cross-reactive with HIV-1 p24 (Figures 7A and 7B). However, unlike the cross-reactive anti-p24 antibodies that developed before other reactive antibodies in the infected cat sera, the cross-reactive anti-p24 antibodies in the vaccinated cat sera were detected after the development of cross-reactive antibodies to Env (gp120, gp160) and polymerase (p66).

In order to negate nonspecific reactivity caused by cellular antigens, selected sera from FIV-vaccinated and FIV-infected cats were preabsorbed extensively with either uninfected feline FeT-J cells or FIV-infected FeT-J cells. The dual-subtype vaccine viruses were produced in FeT-J cells or a cell line from FeT-J lineage (FL-4 cells) (Pu *et al.*, 2001). Those sera preabsorbed with uninfected cells were then competed with uninfected FeT-J cell lysate, while those preabsorbed with FIV-infected FeT-J cells were competed with pelleted FIV preparation. The cross-reactivity with HIV-1 p24, p18, p32, gp41, gp120, gp160, and polymerase p51/p66 were not removed in HIV-1 immunoblots by uninfected cell absorption/competition but were completely removed by FIV-infected cell absorption/competition, demonstrating that the FIV-specific antibodies were reacting specifically with HIV-1 proteins (Figure 8A). As an additional precaution, a separate set of these cat sera (cats #973 and #3L4) were preabsorbed with either uninfected human cells (H9/HuT-78 combination) or HIV-1-infected human T cells (HIV_{LAV}/H9 and HIV_{UCD1}/HuT-78 combination). Only preabsorption with HIV-1 infected cells was able to remove HIV-1 cross-reactivity from the FIV-specific sera (Figure 8B). As an additional confirmation that FIV-specific sera cross-react with HIV-1, these cat sera were evaluated in ELISA for reactivity with recombinant $HIV\text{-}1_{BRU}$ p24 and recombinant $HIV\text{-}1_{IIIB}$ gp160. Both vaccinated cat sera and post-infection sera clearly cross-reacted with $HIV\text{-}1_{BRU}$ p24 and $HIV\text{-}1_{IIIB}$ gp160 (Figure 9A). These results further confirm that the HIV-1 cross-reactivity of FIV-specific cat sera is reacting specifically to HIV-1 protein(s).

As a means of evaluating the importance of cross-reactive antibodies to HIV-1, reactive sera from dual-subtype vaccinated cats were tested for the presence of FIV and HIV-1 neutralizing antibody titers. The dual-subtype FIV vaccine has been shown to induce VN antibodies to homologous and heterologous FIV strains (Pu *et al.*, 2001). Sera from six vaccinated cats (Cats #N55, #973, #C6G, #3L4, #C9K, and #C9F) with strong cross-reactive antibodies to HIV-1 Env were tested in a VN assay using human peripheral blood mononuclear cells (PBMC) as indicator cells and HIV-1_{LAV} or HIV-1_{UCD1} as inocula (Table 2). Controls included pooled serum from two HIV-1 positive individuals, serum from an HIV-negative individual, and sera from SPF cats immunized with uninfected FeT-J cells (Cat #3G5) or Fet-J lysates (Cat #C6E). Both proviral PCR levels and RT activity were used to detect HIV-1 levels in the antibody-treated cultures. One (Cat #C6G) of six vaccine sera tested positive for VN antibodies to HIV-1_{LAV} (50 VN titer) and to HIV-1_{UCD1} (10 VN titer). This sera was the strongest one of the four sera (Cats #C6G, #973, #C9K, and #C9F) that also reacted in the Cambridge HIV-1_{IIIB} immunoblot at gp120 (Table 2, Figures 6B and 7B). These results are indicative of the importance of evolutionarily conserved epitopes in generating cross-reactive and neutralizing antibodies to HIV-1 Env.

Since FIV-specific antibodies react to recombinant HIV-1 p24 protein, it is conceivable that this protein has epitopes for stimulating the necessary cellular immune components such as cytokines needed to develop these cross-reactive antibodies. The ability of FIV/HIV-1 evolutionarily conserved epitopes to generate cellular immunity was evaluated. In previous studies, PBMC from dual-subtype FIV vaccinated cats produced high levels of interferon-γ (IFNγ) production upon stimulation with inactivated whole FIV antigens (Pu *et al.*, 2001; Pu *et al.* 1999). IFNγ is a TH1 cytokine that is also essential in the production of IgG (Abbas *et al.*, 2000). Thus, the ability of PBMC from dual-subtype vaccinated cats to produce IFNγ in response to either HIV-1_{BRU} p24, FIV p24, or inactivated whole FIV stimulations was evaluated (Figure 9B). Equivalent amounts of uninfected FeT-J cells were used as nonspecific control stimulants, while Staphylococcal enterotoxin A (SEA, 1 μg/ml) was used as positive control stimulant. Significant titers of IFNγ (243-2187 U/ml) were

detected upon stimulation with inactivated whole FIV_{Pet} and FIV_{Shi} in all vaccinated cats (Figure 9B). In contrast, IFNγ production in response to stimulation with FIV or HIV p24 proteins was low in two vaccinated cats (#C9X and #C9W) but significant in Cat #C9Y (256 U/ml).

Example 3 - Partial *gag* Sequence Analysis Following Real-time PCR

Partial FIV *gag* were isolated from cocultures of #FH1 PBMC with either human PBMC (B4) or feline PBMC (A9) using Real-time PCR followed by regular PCR. Positive PCR products after Real-time PCR were used as samples for regular PCR. All the primers and probe for Real-time PCR and regular PCR were described in Norway *et al.* (2001). After regular PCR, amplification products of the expected size were isolated from agarose gel and cloned into the pCR 2.1 TOPO cloning vector according to the manufacturer's instructions (Invitrogen, Carlsbad, CA) and sequenced (ICBR core lab, UF) (Figure 10). These results confirm the presence of FIV sequences in the #FH1 subject's cells. The conditions under which the assay was performed minimized any possibility of cross-contamination.

Example 4 - Vaccines

Vaccines compositions of the present invention comprising FIV proteins and peptides, recombinant viral vector-based FIV constructs, attenuated or inactivated FIV viral isolates, and the like, having antigenic or immunogenic properties, can be prepared by procedures well known in the art. For example, such vaccines can be prepared as injectables, *e.g.*, liquid solutions or suspensions. Solid forms for solution in, or suspension in, a liquid prior to injection also can be prepared. Optionally, the preparation also can be emulsified. The active antigenic ingredient or ingredients can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Examples of suitable excipients are water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vaccine can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or adjuvants such as aluminum hydroxide or

muramyl dipeptide or variations thereof. Also, cholera toxin subunit B or other agents which stimulate antibody production at mucosal sites can be used. In the case of peptides, coupling to larger molecules such as KLH or tetanus toxoid sometimes enhances immunogenicity. The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers include, for example, polyalkalene glycols or triglycerides. Suppositories can be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10%, preferably about 1 to about 2%. Oral formulations can include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. These compositions can take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain from about 10% to about 95% of active ingredient, preferably from about 25% to about 70%.

Compositions can be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The quantity to be administered can depend on the subject to be treated and the degree of protection desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and can be peculiar to each individual. However, suitable dosage ranges are of the order of about several hundred micrograms active ingredient per individual. Suitable

regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed in one or two week intervals by a subsequent injection or other administration.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

Table 1. HIV-1 IMMUNOBLOT REACTIVITY OF FIV-INFECTED AND FIV-VACCINATED CAT SERA [a]

Viral Protein	Vaccinated Cat Sera		Infected Cat Sera[a]				
	Dual Subtype	Single Subtype	FIV	Pet	UK8	Bang	Shi
Total Tested	13	12	41	10	11	11	9
Total Positive	13	11	18	3	7	5	3
% Positive	100%	92%	44%	30%	64%	45%	33%
p24	11	6	16	1	7	5	3
p18	8	1	2	0	1	1	0
p55	2	0	4	0	1	1	2
p32	3	0	2	0	2	0	0
p51	2	1	0	0	0	0	0
p66	9	8	2	1	0	0	1
gp41	6	3	4	1	0	1	2
gp120	5	4	2	1	1	0	0
gp160	1	0	1	0	1	0	0

a Nine of 19 (47%) cats infected with in vitro-derived inocula and 9 of 22 (41%) cats infected with in vivo-derived inocula developed antibodies to HIV-1. Hence, both in vitro and in vivo-derived inocula were equally effective at inducing cross-reactive antibodies to HIV-1. Inoculation dose (10-100 CID_{50}) had no statistically significant effect on the frequency or level of HIV cross-reactivity (individual data not shown). Except for the antibodies to gp120 and gp160, a serum was considered antibody positive for specific HIV protein if it was positive on either HIV-1_{IIIB} or HIV-1_{UCD1} immunoblot analysis. A serum was considered positive for either gp120 or gp160 if it was positive on both HIV-1_{IIIB} and HIV-1_{UCD1} immunoblot analyses. Thirteen of 13 dual-subtype vaccinated and 11 of 12 single-subtype vaccinated had either antibodies to gp120 or gp160 in HIV-1_{UCD1} immunoblot analysis (data not shown). Data from a stricter analysis are shown for envelope antibodies because of the presence of antibodies to cellular proteins that reacted close to but clearly different from gp120 and gp160.

Table 2. SUMMARY OF THE SERUM VN TITERS AND SERUM REACTIVITY TO VIRAL ENV

CAT # (TYPE OF IMMUNIZATION)	HIV-1 IMMUNOBLOT (gp120 / gp160)		$HIV\text{-}1_{IIIB}$ gp160 ELISA TITER	VN ANTIBODY TITER TO				
				HIV-1		FIV		
	UCD1	IIIB		UCD1	LAV	Pet	Bang	Shi
C6G (Virus Vaccine)	+ / +	+ / -	>1200	10	50	50	50	>500
C9K (Virus Vaccine)	+ / +	+ / -	1200	<5	5	50	5	500
C9F (Virus Vaccine)	+ / +	+ / -	1200	<5	<5	5	5	500
3L4 (FIV-Cell Vaccine)	+ / +	- / -	1200	<5	<5	>1000	>500	<10
N55 (FIV-Cell Vaccine)	+ / +	- / -	600	<5	<5	100	500	<10
973 (FIV-Cell Vaccine)	+ / +	+ / -	>1200	<5	<5	100	50	<10
3G5 (Uninfected Whole Cell)	- / -	- / -	<300	<5	<5	<5	<5	<5
C6E (Uninfected Cell Lysate)	- / -	- / -	<300	<5	<5	<5	<5	<5
Pooled HIV-Positive Serum	+ / +	+ / +	>1200	>500	50	5	5	5
HIV-Negative Serum	- / -	- / -	<300	<5	<5	<5	<5	<5

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WO 99/60988

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